Tetrahedron Letters 57 (2016) 2219–2221

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Biomimetic synthesis of bis-α-substituent pyrrolidine alkaloids based on a proposed biosynthetic pathway

Zhi Cao, Yueqing Li, Shisheng Wang, Bo Tang, Xiuhan Guo, Liu Wang, Weijie Zhao *

School of Pharmaceutical Science and Technology, State Key Laboratory of Fine Chemicals, Dalian University of Technology, Dalian 116024, PR China

ARTICLE INFO

Article history:
Received 26 December 2015
Revised 27 March 2016
Accepted 31 March 2016
Available online 1 April 2016

Keywords:
Bis-α-substituent pyrrolidine alkaloids
Pyrroloxazine alkaloids
Pyrrolozoxazine
Biosynthetic pathway

ABSTRACT

A possible biosynthetic pathway of bis-α-substituent pyrrolidine alkaloids was proposed. Based on the proposed biosynthetic pathway, six pyrrolozoxazine alkaloids and one *N*-alkyl-5-hydroxymethyl-pyrrole-2-carbaldehyde alkaloid were synthesized. In a mixture of acetic acid and triethylamine, condensation of D-fructose and D-amino acids produced pyrrolozoxazine alkaloids. Replacing D-amino acids with tyramine can afford pyrrolezanthine—a *N*-alkyl-5-hydroxymethyl-pyrrole-2-carbaldehyde alkaloid.

Pyrrolozoxazine alkaloids **1**–**6** (Fig. 1) from natural world were reported successively. Alkaloid **1** was found from *Capparis spinosa* in 2010.[1] Alkaloids **1**–**6** were isolated from the fruits of *Morus alba*[2] in 2014. Alkaloids **2**–**5** with *R*-configuration at C-8 were also found in the fermentation broth of an endophytic actinomycetes.[3] Pyrrolezanthine (**7**), a *N*-alkyl-5-hydroxymethyl-pyrrole-2-carbaldehyde alkaloid, was isolated from Formosan *Zanthoxylum simulans*,[4] mushroom *Leccinum extremiorientale*,[5] *Alhagi sparsifolia*[6] and Actinobacterium *Jiangella gansuensis*.[7] All alkaloids in Figure 1 contain the same substructure of formyl pyrrole. The formyl pyrrole and oxazine are regarded as important pharmacophore units. Alkaloid **6** significantly inhibited pancreatic lipase activity.[2] Acortatarins A bearing formyl pyrrole group and oxazine ring could inhibit reactive oxygen species production in high-glucose-stimulated mesangial cells.[8]

The existence of such kind of alkaloids in nature is interesting for many researchers. We have done the total synthesis of alkaloid **1** in six steps with a 17.0% overall yield,[9] starting from L-alanine. We have been committed to the synthesis of bis-α-substituent pyrrolidine alkaloids, and synthesized two bis-α-substituent pyrrolidine alkaloids, pollenopyrroside A and capparisine B, from D-fructose in 14 steps.[10] The first synthesis of alkaloid **1** was reported in 1971 via roasting DL-alanine and D-glucose at 200–250 °C.[11] Alkaloids **2**, **3**, and **4** were first found in the process of roasting chicory root without any chemical reagent.[12] Alkaloids **5** and **6** were achieved from monosaccharide ester as side products with yields of 11% and 4%.[13] These Letters offered us enlightenment that pyrrolozoxazine alkaloids can be obtained from natural compounds directly. We believed the biosynthesis of bis-α-substituent pyrrolidine alkaloids should be related with D-fructose and primary amines.

Proposed biosynthetic pathway of alkaloids includes ornithine pathway, lysine pathway, anthranilic acid pathway, phenylalanine/tyrosine pathway, and tryptophan pathway. Among them, ornithine pathway is the main origin of α-substituent-pyrrolidine alkaloids. But the biosynthetic pathway of increasing occurrence of bis-α-substituent-pyrrolidine alkaloids, such as acortatarins,[14] pollenopyrroside[15] and capparisine[1] remains unknown. Herein we proposed a possible biosynthetic pathway of bis-α-substituent pyrrolidine alkaloids including pyrrolozoxazine alkaloids **1**–**6** and Pyrrolezanthine **7**.

Maillard reaction of primary amine with D-fructose or D-glucose yielded 5-hydroxymethyl-pyrrole-2-carbaldehyde unit,[16] but it has not been proven occurring in plants yet. We suggested that bis-α-substituent-pyrrolidine alkaloids may come from a type of Maillard reaction of D-fructose and primary amine in plants as Figure 2.

The fact that alkaloids **2**, **3**, and **4** were first found in the process of roasting chicory root without any chemical reagent[12] showed pyrrolozoxazine alkaloids can be obtained from natural compounds directly.

Applying Maillard reaction, 5-hydroxymethyl-pyrrole-2-carbaldehyde unit can be obtained from D-fructose and amino group as Figure 2. The amine part of alkaloids **1**–**6** should be amino acids. The condensation of D-glucose and primary amines has been

* Corresponding author. Fax: +86 0411 84701411.
E-mail address: Pharmscizhao@163.com (W. Zhao).

http://dx.doi.org/10.1016/j.tetlet.2016.03.104
0040-4039/

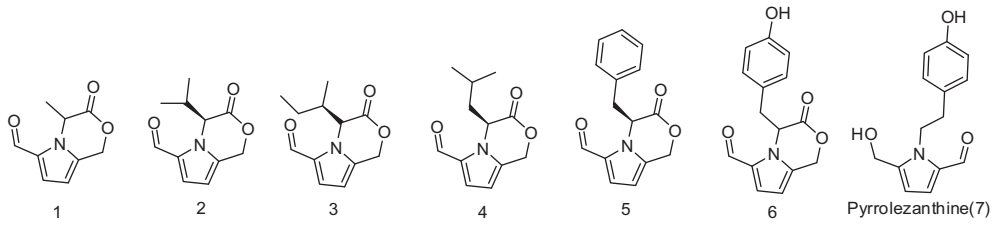


Figure 1. Reported structures of alkaloids **1**–**7**.

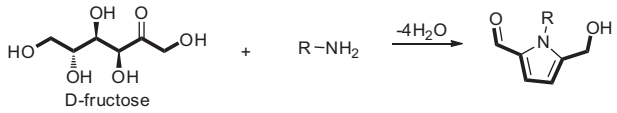
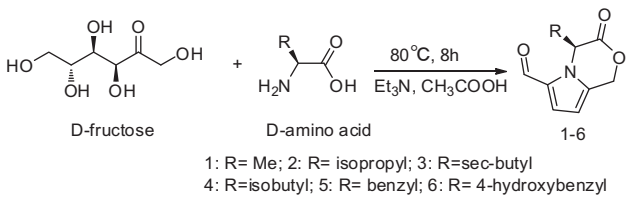


Figure 2. Proposed biosynthesis of alkaloids with 5-hydroxymethyl-pyrrole-2-carbaldehyde unit.

reported in DMSO and oxalic acid.[17] This kind of conversion occurred in acid environment. As Paal–Knorr pyrrole synthesis was conducted in acetic acid, we tried the dehydration in acetic acid from D-fructose and alanine at 80 °C as Scheme 1. Although alkaloids **1** was obtained, the yield was only 2% calculated with alanine (entry 1 in Table 1). The side product 5-hydroxymethylfurfural (5-HMF) and large amount of formed caramel became a barrier of desired alkaloids.

The changes of reaction conditions with lower temperature (entry 2 in Table 1), longer incubation time (entry 3 in Table 1) or D-fructose replaced by D-glucose (entry 4 in Table 1) did not increase the yield. We cut down the usage of acetic acid (entry 5 in Table 1) and found that alkaloid **1** could not be generated. In the mixture of acetic acid and pyridine, the yield had no obvious increments (entry 6 in Table 1). Triethylamine replacing pyridine as component solvent[13] (volume ratio = 2:1) could increase the yield (up to 3.8%). The yield was raised with the augment of D-fructose (1:1–1:5, entries 7–10 in Table 1). When the ratio of acetic acid and triethylamine was changed to 4:3, the yield reached 18.4% (entry 11 in Table 1). The mixture solvent could drastically reduce the side products, including caramel and 5-HMF. So corresponding amino acid and D-fructose (5 equiv) were dissolved in acetic acid and triethylamine (volume ratio = 4:3), then stirred at 80 °C for 5–10 h (monitored by TLC). Alkaloids **2**–**6** were obtained in the yield of 15.1–21.2% (Table 2).

The ^{1}H and ^{13}C NMR spectra data of synthetic products **1**–**6** were identical with those reported data for natural products.[2] In view of the specific rotations, the synthetic products 2–5 ($[\alpha]_D^{20}$ −32 to −88) were consistent with reported data ($[\alpha]_D^{20}$ −32 to −88). The products of this route were enantioenriched while the product by Bu et al. was raceme.[9] The specific rotations of alkaloid **1** found in *Capparis spinosa* was −33.3 (*c* 0.05, MeOH),[1] which was in accordance with our final product ($[\alpha]_D^{20}$ −36 (*c* 0.27, MeOH)) of D-alanine. Otherwise, the specific rotations of **1a** with *S*-configuration at C8, the product of L-alanine was +38 (*c* 0.08, MeOH). So the



Scheme 1. Biomimetic synthesis of alkaloids **1**–**6**.

Table 1
Optimization in the condensation of D-fructose and D-alanine

Entry	Mole ratio[a]	Solvent	*T* (°C)	*t* (h)	Yield[b] (%)
1	1:1	Acetic acid	80	8	2
2	1:1	Acetic acid	50	8	1.2
3	1:1	Acetic acid	50	18	1.3
4	1:1	Acetic acid	80	8	2.1
5	1:1	EtOH, 1.5 equiv acetic acid	80	8	0
6	1:1	Acetic acid + pyridine (2:1)	80	8	2.2
7	1:1	Acetic acid + triethylamine (2:1)	80	8	3.8
8	1:2	Acetic acid + triethylamine (2:1)	80	8	8.2
9	1:3	Acetic acid + triethylamine (2:1)	80	8	8.7
10	1:5	Acetic acid + triethylamine (2:1)	80	8	9.6
11	1:5	Acetic acid + triethylamine (4:3)	80	8	18.4
12	1:6	Acetic acid + triethylamine (4:3)	80	8	18.6

[a] Mole ratio was the mole of D-alanine to D-fructose, except that entry 4 was mole of D-alanine to D-glucose.
[b] Yield was calculated with D-alanine.

Table 2
Yields of alkaloids **1**–**6** and corresponding enantiomeric excess (ee)

Entry	Substrate	Product	Yield (%)	Ee[a] (%)
1	D-Alanine	Alkaloid **1**	18.4	50
2	D-Valine	Alkaloid **2**	16.7	94
3	D-Isoleucine	Alkaloid **3**	15.1	96
4	D-Leucine	Alkaloid **4**	15.7	79
5	D-Phenylalanine	Alkaloid **5**	18.5	95
6	D-Tyrosine	Alkaloid **6**	21.2	93
7	L-Alanine	Compound **1a**	18.4	30
8	D-Alanine methyl ester	Alkaloid **1**	13.8	50

[a] Yield was calculated with amino acid or amino acid methyl ester.

alkaloids **1**–**6** were inferred as *R*-configuration at C-8 based on literature[3] and specific rotations of our final products.

The mechanism of the dehydration of D-fructose and amino acids is proposed in Figure 3. First, D-fructose and amino acids formed Schiff base **B** in acid. Second, the Schiff base could be isomerized to enamine **C**.[17] By eliminating one molecule of H_2O, enamine **C** was converted to intermediate **D**.[18] Then, the nitrogen attacked the C-5 atom to form the pyrrole ring (**E**). Effect of electron pushing of nitrogen atom promoted the removal of 4-hydroxyl group and the aromatization to pyrrolic compound (**F**). Finally, our target products were formed via intramolecular esterification in acid.

We established the ee of products by high performance liquid chromatography (HPLC) analysis employing a Chiralcel OD-H (250 mm × 4.6 mm) column as Table 2. The fact that ee of alkaloids **1** and **1a** were 50% and 30% elucidated the conditions of acetic acid

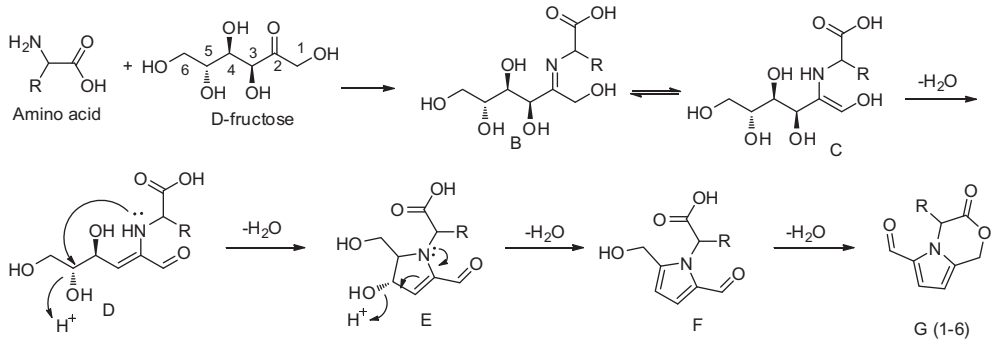
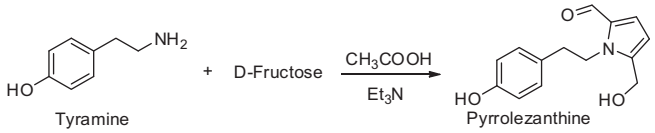


Figure 3. Proposed mechanism of the dehydration of D-fructose and amino acid.



Scheme 2. Biomimetic synthesis of pyrrolezanthine.

and triethylamine led to partial racemization of the stereocenter. But for alkaloids **2**–**6** (except for alkaloid **4**), the ee were 93–96%. There were little enantiomers formed. Based on the Letter before,[19] the formation of labile Schiff bases (B) might be the reason of racemization. And the steric and electronic properties of R group influenced the formation of labile Schiff bases and then affected racemization. The partial racemization provided further evidence that our mechanism via Schiff bases was reasonable.

Maillard reaction of amine and D-glucose or D-fructose can afford bis-α-pyrrolidine alkaloids in a mild condition. As amino acid and D-fructose are natural substance, this way may also be the biosynthetic pathway of bis-α-pyrrolidine alkaloids. To validate the pathway is general or not, pyrrolezanthine **7** was synthesized from D-fructose and tyramine (Scheme 2). In a mixture of triethylamine and acetic acid (volume ratio is 4:3), the yield could reach to 39.7%.

In summary, bis-α-substituent pyrrolidine alkaloids were achieved from natural D-fructose and D-amino acids (or tyramine) in a solution of acetic acid and triethylamine in the yield of 15.1–21.2%. The proposed biosynthetic pathway is suitable for pyrrolezanthine in a 39.7% yield. Proteinogenic amino acids are always L-stereochemistry, whereas natural D-amino acid should be the major biosynthetic precursor of pyrrolozoxazine alkaloids in view of our biomimetic route.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.03.104.

References and notes

1. Yang, T.; Wang, C.-H.; Chou, G.-X.; Wu, T.; Cheng, X.-M.; Wang, Z.-T. *Food Chem.* **2010**, *123*, 705–710.
2. Kim, S. B.; Chang, B. Y.; Hwang, B. Y.; Kim, S. Y.; Lee, M. K. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 5656–5659.
3. Wang, P.; Kong, F.; Wei, J.; Wang, Y.; Wang, W.; Hong, K.; Zhu, W. *Marine Drugs* **2014**, *12*, 477–490.
4. Yang, Y.-P.; Cheng, M.-J.; Teng, C.-M.; Chang, Y.-L.; Tsai, I.-L.; Chen, I.-S. *Phytochemistry* **2002**, *61*, 567–572.
5. Choi, J.-H.; Ozawa, N.; Yamakawa, Y.; Nagai, K.; Hirai, H.; Kawagishi, H. *Tetrahedron* **2011**, *67*, 6649–6653.
6. Zou, G. A.; Mansur, S.; Hu, S. C.; Aisa, H. A.; Shakhidoyatov, K. M. *Chem. Nat. Compd.* **2012**, *48*, 635–637.
7. Han, L.; Gao, C.; Jiang, Y.; Guan, P.; Liu, J.; Li, L.; Xu, L.; Huang, X. *J. Nat. Prod.* **2014**, *77*, 2605–2610.
8. Zhao, Z.-F.; Zhou, L.-L.; Chen, X.; Cheng, Y.-X.; Hou, F.-F.; Nie, J. *Chin. Med. J.* **2013**, *126*, 1230–1235.
9. Bu, X.; Li, Y.; Liu, J.; Zeng, D.; Zhao, W. *Chem. Nat. Compd.* **2012**, *48*, 194–197.
10. Cao, Z.; Li, Y. Q.; Wang, S. S.; Guo, X. H.; Wang, L.; Zhao, W. J. *Synlett* **2015**, 921–926.
11. Shigematsu, H.; Kurata, T.; Kato, H.; Fujimaki, M. *Agric. Biol. Chem.* **1971**, *35*, 2097–2105.
12. Sannai, A.; Fujimori, T.; Kato, K. *Agric. Biol. Chem.* **1982**, *46*, 429–433.
13. Jerić, I.; Šimici, L.; Stipetić, M.; Horvat, S. *Glycoconjugate J.* **2000**, *17*, 273–282.
14. Tong, X.-G.; Zhou, L.-L.; Wang, Y.-H.; Xia, C.; Wang, Y.; Liang, M.; Hou, F.-F.; Cheng, Y.-X. *Org. Lett.* **2010**, *12*, 1844–1847.
15. Guo, J. L.; Feng, Z. M.; Yang, Y. J.; Zhang, Z. W.; Zhang, P. C. *Chem. Pharm. Bull.* **2010**, *58*, 983–985.
16. (a) Kato, H.; Sonobe, H.; Fujimaki, M. *Agric. Biol. Chem.* **1977**, *41*, 711–712; (b) Antal, M. J., Jr; Mok, W. S. L.; Richards, G. N. *Carbohydr. Res.* **1990**, *199*, 91–109.
17. Adhikary, N. D.; Kwon, S.; Chung, W.-J.; Koo, S. *J. Org. Chem.* **2015**, *80*, 7693–7701.
18. (a) Román-Leshkov, Y.; Chheda, J. N.; Dumesic, J. A. *Science* **2006**, *312*, 1933–1937; (b) Moreau, C.; Durand, R.; Razigade, S.; Duhamet, J.; Faugeras, P.; Rivalier, P.; Ros, P.; Avignon, G. *Appl. Catal. A* **1996**, *145*, 211–224.
19. Brückner, H.; Justus, J.; Kirschbaum, J. *Amino Acids* **2001**, *21*, 429–433.